Effect of Bovine Serum Albumin on Red Blood Cell Optical Anisotropy Probed Through the Optomechanical Response in an Optical Trap

*Parthasarathi Praveen, Selvan Rekha, Devarkonda Chetana, Shruthi S. Iyengar, Sarbari Bhattacharya, and Sharath Ananthamurthy**

The dynamics of trapped entities in an Optical Trap (OT) can yield information with regards to their viscoelastic response as well as optical anisotropy, if any. Detailed analysis of such dynamics correlated with parameters which affect the response can yield additional clues to the exact effect of these on the trapped entities. In this work, we illustrate this point by showing how the altered behavior of Red Blood Cells (RBC) treated with Bovine Serum Albumin (BSA) yields information about the nature of action of BSA, on which there is no current consensus in literature. We conclude from our studies that BSA treatment leads to a change in the birefringence of the RBCs, a conclusion arrived at from the altered optomechanical response of such cells in a linearly polarized Gaussian beam OT. Furthermore, we argue that the observed changes in cellular optical anisotropy may be thought of as due to changes in the curvature of the RBC membrane. We also note that BSA action could help mimic pathological conditions that result in an altered cell shape.

1. Introduction

When an asymmetrical microstructure is trapped in a linearly polarized OT, it reorients such that it maximizes its volume along the region of highest electric field.[1] Therefore by measuring the reorientation time for a viscoelastic material such as a human RBC in a linearly polarized OT, it is possible to gauge its elastic modulus.[2] The reorientation dynamics of trapped objects become more interesting when trapped entities possess optical anisotropy along with anisotropy of the shape. In a previous work, we showed that the optomechanical response of a trapped RBC in a linearly polarized OT can be used to obtain estimates of its birefringence[3] and that such estimates of RBC birefringence corroborate with direct measurements carried out using a polarizing microscope. Furthermore, in Ref. [3], we also show by means of a geometric model, that the observed variation of birefringence along the RBC diameter may be attributed to the orientation of phospholipid molecules in the lipid bilayer and that any change in the membrane curvature results in an altered value of birefringence. It is thus plausible that pathological conditions and diseases that alter the shape of the RBC will reflect in a change in RBC birefringence. Studies of RBC birefringence could also provide valuable clues to the association of biological functions and physical properties of these cells to their shape.

While earlier work[4–6] on BSA treatment of RBCs suggest that the cell shape is affected, Refs. [4] and [5] additionally report that BSA replaces certain lipids from the outer leaflet of the membrane, whereas Ref. [6] reports that BSA attaches onto certain specific sites on the membrane. Therefore, it becomes important to understand if treatment with BSA removes or adds proteins to the lipid bilayer. Traditionally, such studies have involved sophisticated biochemical protocols and thus were accessible only to specialists working in those areas. Furthermore, BSA is widely used in preparation of RBC samples for in vitro studies to enable retention of their bi-concave shape as well as to prevent adhesion to glass slides.[7,8] Changes wrought about by the presence of BSA on various parameters like optical anisotropy of the cells, their viscoelastic properties or shape must be taken into account when interpreting the results of these studies. Thereby, it is important to know the difference made in the optical properties of RBC when treated with BSA. In this work, we show as a proof of principle that the changes induced in the red cell shape through a treatment with BSA can be understood through an analysis of the realignment process of the RBC slow axis with the polarization direction of the trapping laser beam altered through a rotation of a half-wave plate aligned to the optical path. Furthermore, we show that a treatment with BSA increases the value of the optical anisotropy, a fact which may add credence to the claims made in Ref. [6].

P. Praveen, S. Rekha, D. Chetana, S. S. Iyengar, S. Bhattacharya, S. Ananthamurthy
Department of Physics
Jnanabharathi Campus
Bangalore University
Bangalore 560056, India
E-mail: asharath@gmail.com

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2. Experimental Section

Details of the experimental setup appear elsewhere.[2] About 30 μl of blood drawn from a healthy donor using a finger-prick method was suspended in 0.5 ml of Phosphate Buffer Saline (PBS). This sample was centrifuged at 1000 RPM for 10 minutes. After removing the supernatant, the reminder was washed with either PBS or PBS containing BSA at a concentration of 1.75 mg/ml (BSA-PBS) and was subsequently suspended in BSA-PBS.

About 20 μl of RBC suspended in PBS and BSA-PBS were taken in separate sample holders made by sticking an o-ring of diameter 1 cm and thickness 1 mm on a glass slide. These samples were further diluted with 300 μl of their own suspensions and another glass slide was placed on top so as to make the arrangement air-tight.

The laser power at the sample was kept at 14 mW and a camera set to record videographs at 15 frames per second was turned on before trapping an RBC. A sequence of images in **Figure 1** shows the trapping of an RBC. It must be noted here that cells suspended in both PBS and BSA-PBS appear to maintain their biconcave shape when imaged through our camera with a 100× magnification made possible by the trapping objective.

After trapping an RBC, we rotate the plane of polarization of the trapping laser beam by introducing a half-wave plate in the optical path. The trapped RBC now rotates about the laser propagation direction till it aligns its slow axis which we identified to be along a diameter[3] to the electric field direction. We show this process in **Figure 2**. Subsequently, the videographs are analysed using ImageJ to obtain the angle between the polarization direction and the RBC as a function of time.

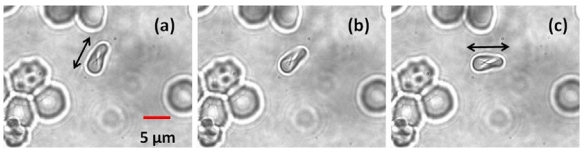

Figure 2. Reorientation of trapped RBC using a λ/2 plate. In frame (a) the electric field makes an angle of 80° and in frames (b) and (c) the electric field direction is along the horizontal. The double sided arrows indicate the direction of the electric field. The laser propagation direction and direction of viewing are both the same and are perpendicular to the image plane.

3. Results and Discussion

According to Ref. [9], the equation of motion of an RBC reorienting under the influence of torque due to birefringence may be written as

$$\alpha\frac{32}{3}\eta r^3\dot{\theta} = -\frac{P}{\omega}Sin2\theta.\ Cos2\phi.\ Sin(360.\ R/\lambda) \quad (1)$$

where θ is the instantaneous angle between the RBC slow-axis and the Electric field direction, 32/3 is the Perrin co-efficient of a circular disc of radius 'r' suspended in a fluid of static viscosity 'η'. Since a human RBC is a biconcave disc, the Perrin co-efficient needs to be modified from its value of 32/3, which is the value for a circular disc. We subsume this deviation in the Perrin coefficient into the parameter α so that α(32/3) represents the Perrin coefficient for the RBC in question. We draw the attention of our readers to Ref. [2] for a detailed description of the determination of α. ϕ is the degree of ellipticity which is zero for linearly polarized light, P is the laser power, ω the angular frequency of the laser and λ its wavelength. R is the birefringence induced retardation in the laser light propagating through the RBC.

We use $\eta = 0.96 \times 10^{-3}$ poiseuille for water, r = 4 μm as the RBC radius, P = 14 mW, $\omega = 1.77 \times 10^{15}$ Hz and laser wavelength λ = 800 nm in water.

The values of θ obtained from image analysis are plotted against the time of recording and the data is fit to the solution of the equation of motion by keeping the retardation R as a fitting parameter. These plots and their fits for the two varieties of cells studied are shown in **Figure 3**. The mismatch between theoretical fits and experimental data might be attributed to the following: (i) variations in the exact RBC shape and morphology leading to a value of the drag which might be slightly different from the theoretically assumed value of α × (32/3) (ii) The red cell radius need not strictly be 4 microns as assumed by us while obtaining theoretical fits (iii) The trapped RBC deforms as it realigns and as optical anisotropy is dependent on lipid molecule orientation, minor changes in birefringence as cell realigns can be expected which will lead to second order effects which have not been considered here.

The value of retardation obtained for cells not treated with BSA is 1.94 ± 0.32 nm whereas for cells treated with BSA, we obtain R = 3.77 ± 0.13. This enhancement in birefringence may be thought of as due to a slight flattening of the central crater or the dimple region of the RBC which according to the model proposed by us in Ref. [3] brings more phospolipid molecules into parallel alignment with each other. Thus, it may be seen that

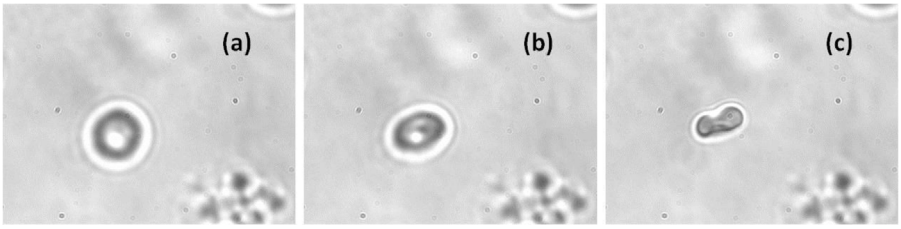

Figure 1. Sequence of images showing the process of reorientation of an RBC about a diameter parallel to its plane. The laser propagation direction and the direction of viewing are both perpendicular to the image plane.

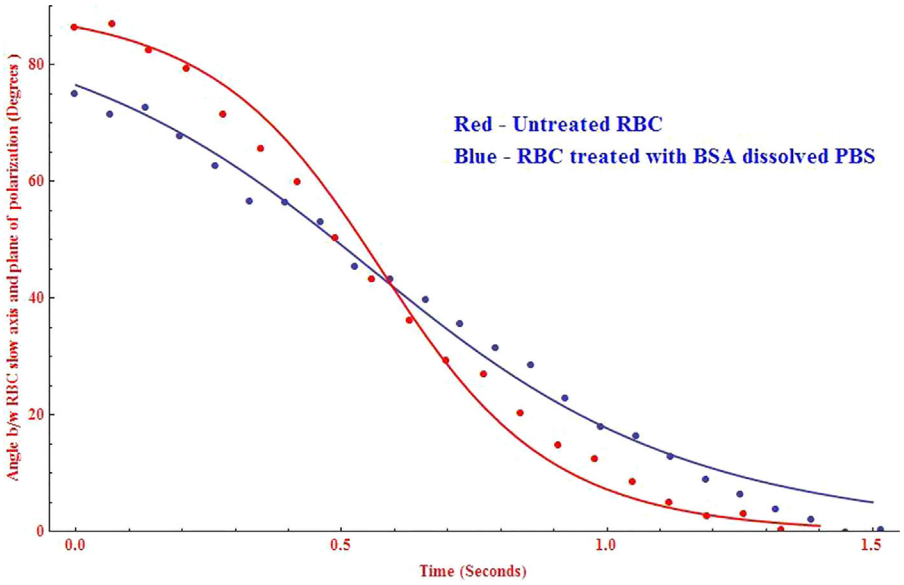

Figure 3. Plot of angle between the RBC Slow-axis as a function of time. Red dots are data of untreated RBC and blue dots are data of RBC treated with PBS containing dissolved BSA at 1.75 mg/ml. The Red and Blue Solid lines are their respective fits. Note that the two cells start reorienting with different initial angles. The untreated cell makes an initial angle of 84° and the BSA treated cell makes an initial angle of 75° with the direction of electric field.

treatment with BSA induces changes in cell shape, leading to changes in the orientation of phospholipid molecules. This in turn, leads to a change in the birefringence of the cell, which we detect by means of the optomechanical response in a linearly polarized OT. Birefringent cell samples may be thus be studied, by means of their optomechanics in an OT leading to a better understanding of the consequent biological and physical properties of the cell.

4. Conclusions and Outlook

We show that changes in optical anisotropy of RBCs brought about by a treatment with BSA can be estimated by studying their optomechanical response in an OT. Since our earlier studies[3] with normal RBCs as well as those RBCs that are subjected to hyperosmotic shock have proved that the observed birefringence is strongly linked to the ordering of the phospholipids in the RBC membrane, enhancement of birefringence with BSA treatment leads us to believe that the lipid bilayer in the RBC membrane must be largely undisrupted by BSA. This work can be taken forward to study the variation of birefringence with BSA concentration and the theoretical model proposed in Ref. [3] may be extended to estimate the changes in the dimensions of the central crater region or the dimple region in an RBC.

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Keywords

birefringence, membranes, optical trap, phospholipids, red blood cell

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